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Drosophila long-chain acyl-CoA synthetase acts like a gap gene in embryonic segmentation

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ABSTRACT

Long-chain acyl-CoA synthetases (ACSLs) convert the long chain fatty acids to acyl-CoA esters, the activated forms participating in diverse metabolic and signaling pathways. dAcsl is the *Drosophila* homolog of human ACSL4 and their functions are highly conserved in the processes ranging from lipid metabolism to the establishment of visual wiring. In this study, we demonstrate that both maternal and zygotic *dAcsl* are required for embryonic segmentation. The abdominal segmentation defects of *dAcsl* mutants resemble those of gap gene *knirps (kni)*. The central expression domain of Kni transcripts or proteins was reduced whereas the adjacent domains of another gap gene Hunchback (Hb) were correspondingly expanded in these mutants. Consequently, the striped pattern of the pair-rule gene Even-skipped (Eve) was disrupted. We propose that *dAcsl* plays a role in embryonic segmentation at least by shifting the anteroposterior boundaries of two gap genes.

Introduction

In *Drosophila* embryo, a hierarchy of maternal, gap, pair-rule and segment polarity genes which encode transcription factors establish the anteroposterior axis and the embryonic segmentation (St Johnston and Nusslein-Volhard, 1992). The spatially restricted transcription factors determine the complex gene expression patterns in the early embryo. Along with the maternal determinants, the gap gene products specify the boundaries of the adjacent gap gene expression domains and the downstream pair-rule gene stripes (Hulskamp et al., 1990; Pankratz et al., 1990; Struhl et al., 1992). Among them, Knirps (Kni) and Hunchback (Hb) form their expression patterns partly through mutual repression (Clyde et al., 2003; Yu and Small, 2008).

The known maternal effectors are not sufficient to establish the gap domains and it is likely that unidentified maternal molecules exist and modulate the gap gene expression (Jaeger et al., 2007). The abundant maternally-deposited lipids in embryos have been recognized as an energy source for early embryo development. These molecules also have important functions in diverse signaling pathways during larval development such as shaping morphogen gradients (Eaton, 2008; Hausmann et al., 2007). However, it remains unclear whether lipids participate in any way in the establishment of embryonic segmentation.

Long chain acyl-CoA synthetase (ACSL) is a family of enzymes which adds Coenzyme A to the long chain (C12-20) fatty acids (Soupene and Kuypers, 2008). As the activated form of fatty acids, the Acyl-CoA participates in various cellular processes including lipid metabolism, vesicle trafficking and signal transduction. ACSL4 is a member of the mammalian ACSL family and its mutations have been associated with non-syndromic X-linked mental retardation (MRX) (Longo et al., 2003; Meloni et al., 2002; Piccini et al., 1998). The *Drosophila* gene *dAcsl* encodes the homolog of human ACSL4 and they are functionally conserved ranging from building visual circuitry to lipid homeostasis (Zhang et al., 2009). However, the developmental function of *dAcsl* at the embryonic stages remains unexplored.

In this report, we illustrate that *dAcsl* is required for embryonic segmentation both maternally and zygotically. The impaired segmentation caused by *dAcsl* mutations is similar to that of gap gene *kni* mutants. In *dAcsl* mutants, the domain of Kni transcripts or proteins was reduced whereas the domain of another gap gene Hb protein was correspondingly expanded. Consequently, the pair-rule gene expressions were perturbed in these embryos. We propose that *dAcsl* participates in embryonic segmentation by spatially modulating gap gene expression.

Materials and methods

Genetics and stocks

dAcsl^{1}, *dAcsl*^{8}, *Df(2R)H3E1*, *knirps*^{1}, *Kruppel*^{1}, *hunchback*^{12}, and *Lsd2*^{KG00149} (*Lsd2*^{KG}) were obtained from Bloomington Stock Center; *PBac(RB)dAcsl*^{e02676} and *PBac(WH)dAcsl*^{f02764} from Harvard collection; *Lsd2*^{NP0141} from Kyoto *Drosophila* Genetic Resource Centre. *dAcsl*^{KO} was generated by precisely deleting the DNA sequence between *PBac(R)*

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dAcsl^{e02676} and *PBac(WH)dAcsl*^{f02764}, removing nearly all *dAcsl* coding sequence except for its first 29 nucleotides. All crosses were done at 25 °C unless specified. Molecular information of allele *dAcsl*^{1} and *dAcsl*^{8} has been described (Zhang et al., 2009). To get the maternal *dAcsl* mutants, we generated the homozygous germ line clones using the dominant female sterile technique (Chou et al., 1993; Chou and Perrimon, 1996). The pupae were heat shocked in 37 °C incubator for an hour and twice each day with an interval of ~10 hours. The virgin females of *hsFLP; FRT 42B ovo*^{D}*/FRT 42B dAcsl*^{1} and *hsFLP; FRT 42B ovo*^{D}*/FRT 42B dAcsl*^{8} were crossed to *dAcsl*^{1}*/CyO Kr>GFP, dAcsl*^{8}*/CyO Kr>GFP*, or *dAcsl*^{KO}*/CyO Kr>GFP*. The embryos were collected and allowed to develop until the 1st instar (L1) larvae. The maternal and zygotic mutants were distinguished by GFP marker. The *dAcsl M*$^{-}$ embryos were the progenies of heat-shocked females of *hsFLP; FRT42B ovo*^{D}*/FRT42B dAcsl*^{1} mated to *dAcsl*^{KO}*/CyO GFP*. These embryos included two zygotic genotypes which were undistinguishable before Stage 7.

In situ hybridization

Digoxigenin-labeled sense and anti-sense RNA probes from dAcsl cDNA clone (LP07340) or clones containing PCR-amplified *kni* cDNA sequence. Whole-mount in situ hybridizations to embryos were performed as described (Tautz and Pfeifle, 1989). The stained embryos were mounted in 70% glycerol and photographed using Zeiss AxioPlan2 microscopic system (DIC objectives and AxioCam MRm or ProgRes C5 cool camera).

Immunostaining

All samples were fixed and stained according to the standard embryo antibody staining protocol (Patel, 1994). Primary antibodies were used at the following dilutions: guinea pig anti-Hb at 1:300, guinea pig anti-Kni at 1:300, guinea pig anti-Kruppel (Kr) at 1:300 (these are from Asian Distribution Center for Segmentation Antibodies, National Institute of Genetics, Japan) (Kosman et al., 1998); Rabbit anti-Bcd at 1:500 (from Gary Stuhl); Rabbit anti-Nos at 1:2000 (from Dahua Chen); mouse anti-Eve (2B8, DSHB) at 1:100. Guinea pig anti-dAcsl antibody was used at a 1:1000 dilution for immunostaining and the specificity of this antibody has been described previously (Zhang et al., 2009). AlexaFluor conjugated secondary antibodies (Molecular Probes of Invitrogen) were used at 1:4000. Fluorescent images were collected by Zeiss ApoTome or Olympus FV1000 Confocal microimaging system.

Cuticle preparation

The embryos of specific genotypes were collected and allowed to develop until the L1 larvae, then placed in the Hoyer's medium and heated at 60 °C for more than 2 hours or until they became clear. The embryos with defective segmentation referred to those having one or more segments loss, or partial loss with segment fusions.

Quantitative analysis of gap gene stripes

The Kni and Hb domains referred to those having visible Kni and Hb nuclear staining. They were outlined and measured using the NIH Image J program and normalized against the total image area of each embryo. For all experiments, *p* values are the results of Student's *t*-test provided by Microsoft Excel (*$p<0.05$; **$p<0.01$). The error bar represents the standard error of mean.

Results

dAcsl is maternally deposited and ubiquitously expressed in the early embryos

Our previous work has shown that *dAcsl* is ubiquitously expressed in the larval tissues and enriched in the ER (Zhang et al., 2009). To evaluate how dAcsl is expressed in the embryonic stages, we examined its expression at mRNA and protein levels. By in situ hybridization, we found that *dAcsl* mRNA is maternally deposited (Fig. 1A and B). Using a

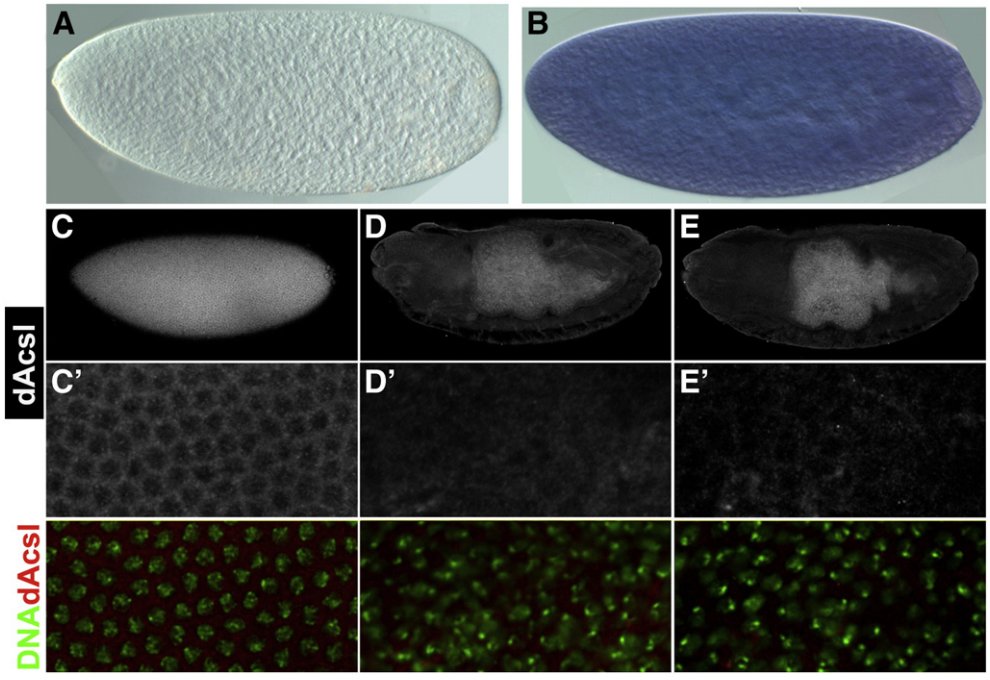

Fig. 1. *dAcsl* is maternally deposited and ubiquitously expressed in early embryos.(A and B) Syncytial blastoderm embryos hybridized with sense (A) or anti-sense (B) *dAcsl* RNA probes. Since the anti-sense probe was generated using the identical region of all *dAcsl* isoforms, it did not distinguish the expression of different isoforms. (C to E') The ubiquitous expression of dAcsl detected by anti-dAcsl antibody, whose specificity in embryo is shown by the strong staining in the wild type cellularized embryo (C and C'), weak staining in the wild type Stage 16 embryo (D and D'), and poor staining in the Stage 16 embryo of *dAcsl*^{KO} (E and E'). Images in C–E or in C'–E' were taken at the same confocal settings. Images in C'–E' are the higher magnifications of the embryos in C–E, respectively. The focal planes for the sections in C'–E' were revealed by the DNA co-staining in the bottom panels.

dAcsl antibody, we demonstrate that dAcsl is cytoplasmically localized and ubiquitously expressed in the embryos (Fig. 1C and D).

The dAcsl mutants exhibited embryonic segmentation defects

In the process of exploring the developmental function of *dAcsl*, we analyzed the larval cuticle patterns and observed the segmentation defects. *dAcsl*^{1} carries an EMS-induced point mutation changing a Val to Asp, which is located within a stretch of highly conserved amino acids near the fatty acyl-CoA synthetase signature motif (Zhang et al., 2009). The more severe allele *dAcsl*^{8} contains three point mutations including K294E, Q419R, and W685stop. K294E is located in the AMP-binding domain and W685stop removes the last 33 amino acids (Zhang et al., 2009). In the *dAcsl*^{1}*/Df(2R)H3E1* embryos, 12% (58 out of 480) displayed the segmentation defects such as segment fusion or deletion, with the segments A2–A7 most frequently affected, and in *dAcsl*^{8}*/ Df(2R)H3E1* embryos, 18% (75 out of 412) were affected (Fig. 2B and C). These results indicate that zygotic *dAcsl* is partially required for embryonic segmentation.

To examine whether the maternal supply of *dAcsl* contributes to the embryonic segmentation, we generated the germ line clones of *dAcsl* mutations. The clonal cells of *dAcsl* strong alleles hardly survived, and we could only use *dAcsl*^{1} and *dAcsl*^{8}. Among the *dAcsl*^{1} maternal mutants, 11.3% (18 out of 155) showed partial deletion or fusion of the abdominal segments (Fig. 2D and G); removal of the zygotic contribution enhanced the phenotypic percentage to 15.3% (19 out of 124) (Fig. 2E and G). More severely, 59% (65 out of 111) of *dAcsl*^{8} maternal mutant embryos exhibited abnormal segments (Fig. 2F and G). In both cases, the segments A1–A7 were the most frequently affected (Fig. 2D–F). This phenotype is similar to that of *kni* mutants, in which the thorax and tail regions developed normally whereas the seven abdominal segments (A1–A7) were missing or fused (Arnosti et al., 1996; Nauber et al., 1988; Nusslein-Volhard et al., 1987). We also found the typical *kni* phenotype in an extreme case of *dAcsl*$^{1}M^{-}$ embryos, the A1–A7 segments were fused together and only A8 remained (Fig. 2E).

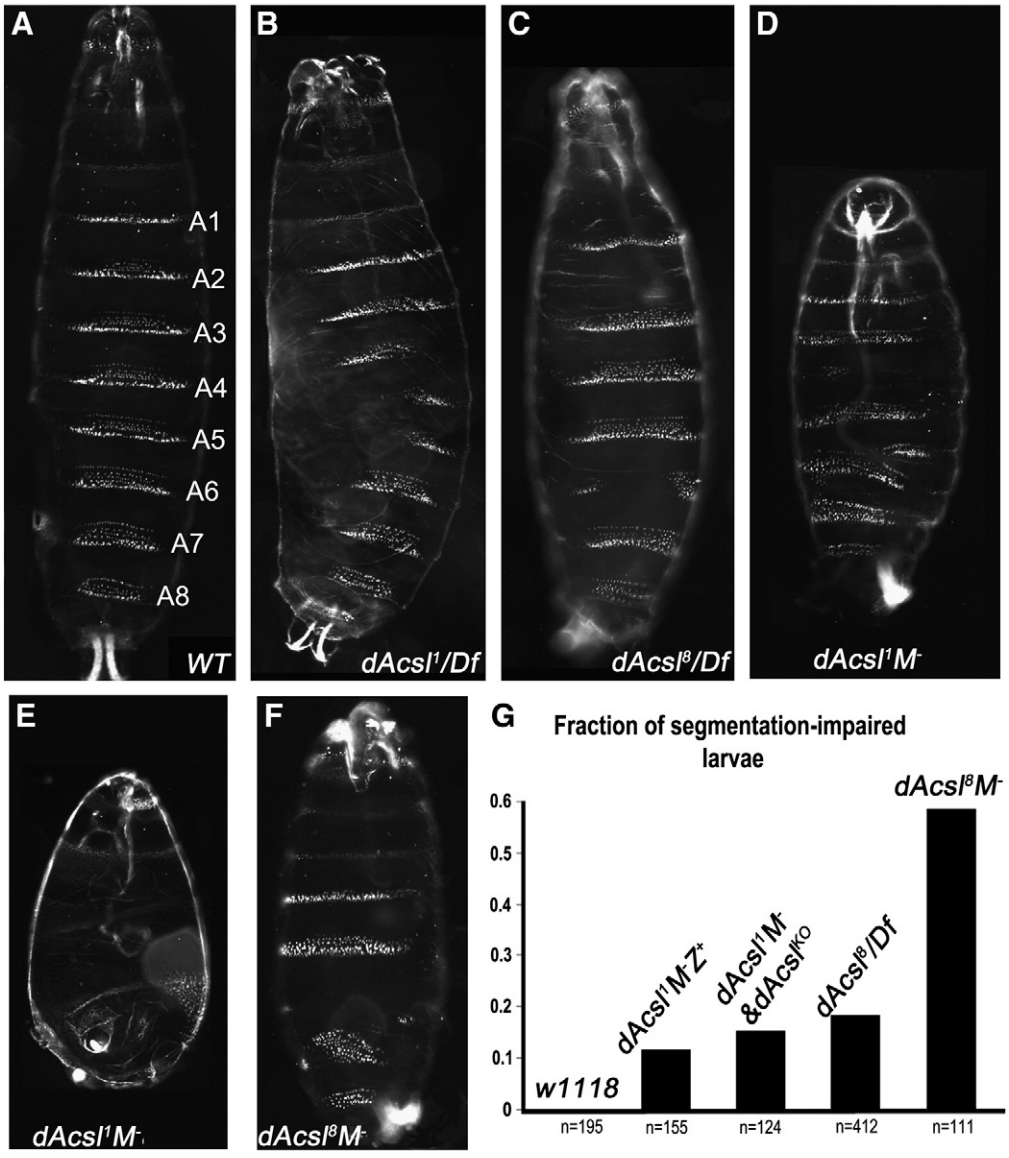

Fig. 2. Both the maternal and zygotic *dAcsl* are required for embryonic segmentation.(A) A wildtype L1 larva. Abdominal segments 1–8 are indicated. (B–F) The segmentations were disrupted in the *dAcsl* mutant larvae. Variable numbers of A1–A7 segments were missing or fused in the *dAcsl* zygotic mutants (B and C), or in the maternal *dAcsl* mutants (D–F). *dAcsl M*$^{-}$ larvae were the progenies of heat-shocked females of *hsFLP; FRT42B ovo*^{D}*/FRT42B dAcsl* mated to *dAcsl/CyO*, and these larvae were not distinguished by their zygotic genotypes. There were also variable defects in the larval head of *dAcsl* mutants. (G) Fraction of segmentation-impaired L1 larvae in different genotypes. The total number of larvae examined for each genotype is listed as '*n*.' None of the 195 *w1118* embryos exhibited defective segmentation. *dAcsl*^{1} *M*$^{-}$*Z*$^{+}$, the larvae from maternal mutants and carrying one zygotic copy of wild type gene; *dAcsl*^{1} *M*$^{-}$and *dAcsl*^{KO}, the larvae of maternal and zygotic mutant. The zygotic genotype was determined by a GFP marker at late embryonic stage.

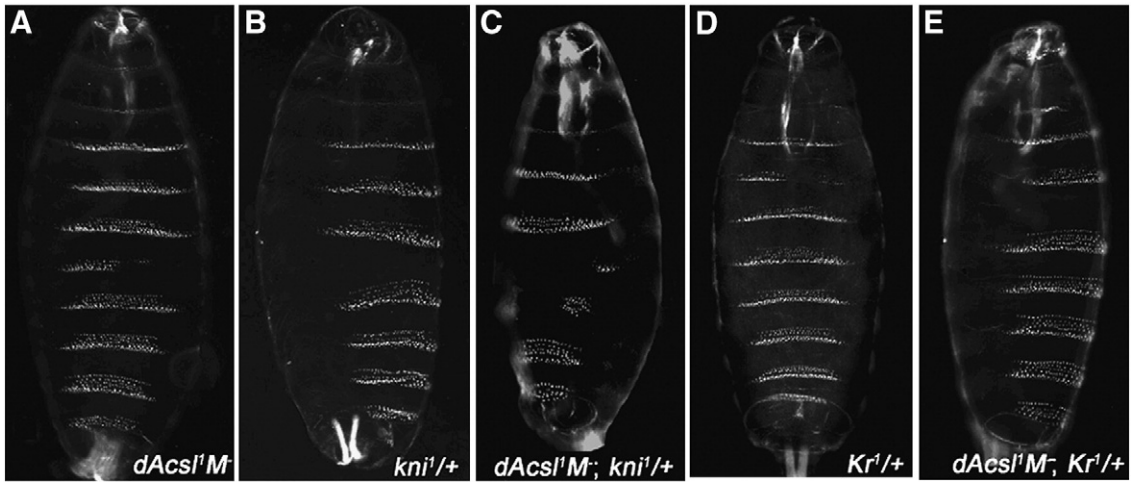

Fig. 3. *dAcsl* genetically interacts with *kni*.Larval cuticle preparations revealed segmentations. *dAcsl*$^{1}M^{-}$ embryos were the progenies of heat-shocked females of *hsFLP; FRT42B ovo*^{D}*/FRT42B dAcsl*^{1} mated to *w1118* males. *dAcsl*$^{1}M^{-}$; *kni*^{1}/+ or *dAcsl*$^{1}M^{-}$, *Kr*^{1}/+ were obtained from crosses of heat-shocked females of *hsFLP; FRT42B ovo*^{D}*/FRT42B dAcsl*^{1} mated to *kni*^{1}*/TM6B GFP* or *Kr*^{1}*/CyO GFP* males respectively. *kni*^{1}/+ and *Kr*^{1}/+ were obtained from the crosses of the *w1118* females mated to *kni*^{1}*/TM6B GFP* and *Kr*^{1}*/CyO GFP* males respectively. GFP or non-GFP L1 larvae were selected for examination.

Taken together, both maternal and zygotic *dAcsl* mutants exhibited segmentation defects similar to that of *kni* mutants.

The segmentation impairment of dAcsl was strongly enhanced by kni mutation

Since *dAcsl* mutants displayed abnormal segmentation similar to the gap gene *kni* mutant phenotype, we explored the potential interaction between *dAcsl* and *kni*. We examined the phenotypic change by introducing *kni*^{1} into the *dAcsl*$^{1}M^{-}$ mutant background. We did not score the embryos which lost only A4 segment because *kni*^{1}/+ embryos themselves displayed a high frequency of A4-loss (data not shown). When the *dAcsl* maternal mutants were combined with one copy of *kni*^{1}, the fraction showing defective segmentation was significantly increased from 12% to 70% (Table 1). Meanwhile, the segmentation abnormality became more severe, indicated by more segments being disrupted in each mutant embryo (Fig. 3C). We also tested the genetic interactions between *dAcsl* and other gap gene mutants including *Kr* and *hb*. The *Kr* mutations also enhanced the percentage of segmentation-impaired embryos although the severity was not obviously changed (Fig. 3E and Table 1). Mutations of another gap gene *hb* did not display a detectable change to the phenotypic

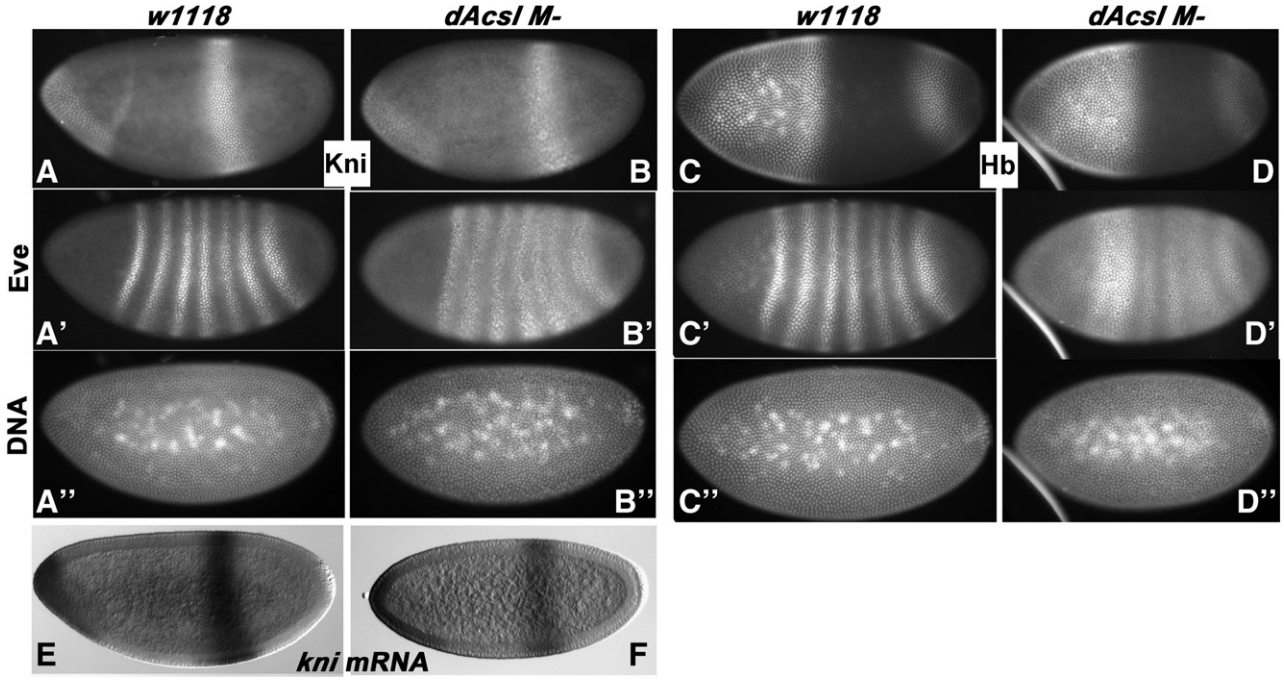

Fig. 4. The 7-strip of pair-rule gene *eve* was not well resolved in the *dAcsl* maternal mutant embryos.The *dAcsl M*$^{-}$ embryos were the progenies of heat-shocked females of *hsFLP; FRT42B ovo*^{D}*/FRT42B dAcsl*^{1} mated to *dAcsl*^{KO}*/CyO GFP*. These embryos included two zygotic genotypes which were undistinguishable before Stage 7. All embryos were immunostained (except E and F) and the images were taken at the same magnification. Embryos in panels A–A", B-B", C–C", or D–D" are the same ones co-stained for Kni or Hb, Eve and DNA. They were cellularized embryos judged by DE-cadherin labeling (data not shown). Embryos in E and F were in situ hybridized with *kni* anti-sense RNA probes.

Table 1
dAcsl genetically interacts with *kni* and *Kr*.

Genotype	Fraction of embryos with defective segments	Embryos examined
w1118	0	195
$kni^1/_+$	5%[a]	190
$dAcsl^1M^-$	12%	209
$dAcsl^1M^-$; $kni^1/_+$	70%[a]	237
$dAcsl^1M^-$; $hb^{12}/_+$	14%	241
$dAcsl^1M^-$; $Kr^1/_+$	50%	234
$Kr^1/_+$	15%	210
$Lsd2^{KG}$	3%	200
$Lsd2^{NP0141}$	1%	264

$kni^1/+$ and $Kr^1/+$ were obtained from the crosses of the *w1118* females mated to $kni^1/TM6B\ GFP$ and $Kr^1/CyO\ GFP$ males, respectively. $dAcsl^1M^-$ embryos were the progenies of heat-shocked females of *hsFLP; FRT42B* ovo^D/*FRT42B* $dAcsl^1$ mated to *w1118* males. $dAcsl^1M^-$; $kni^1/+$ and $dAcsl^1M^-$ $Kr^1/+$ were pregenies of heat-shocked females of *hsFLP; FRT42B* ovo^D/*FRT42B* $dAcsl^1$ mated to $kni^1/TM6B\ GFP$ and $Kr^1/CyO\ GFP$ males, respectively. Note the penetrance of defective segmentation was significantly enhanced from 12% to 70% in the $dAcsl^1M^-$;$kni^1/+$ mutant embryos compared to that of $dAcsl^1M^-$.

[a] When doing the genetic interaction tests between *kni* and *dAcsl*, we did not consider embryos missing A4 segment (a feature of $kni^1/+$) as 'with defective segments.' For example, the 5% $kni^1/+$ embryos with defective segmentation did not include embryos missing A4. $Lsd2^{KG}$ and $Lsd2^{NP0141}$ are homozygous viable and the embryos examined were the progenies of the homozygous mutants.

severity of *dAcsl* mutants (Table 1 and data not shown). These results suggest that *dAcsl* is involved in the gap gene function of *kni* and to a lesser extent of *Kr*.

The Hb and Kni domains were altered in dAcsl mutant embryos

Since *dAcsl* mutant embryos phenocopied *kni* mutants and the segmentation defects were strongly enhanced by *kni* mutations, then does *dAcsl* regulate the expression or function of *kni*? To answer this question, we examined the expression patterns of pair-rule genes (the downstream targets of the gap genes) and the gap genes. In the wild type late Cycle 14 embryos, seven Eve stripes were evenly distributed (Fig. 4A' and C'). In about 40%(110 embryos examined)of the $dAcsl^1M^-$ embryos, one or more Eve stripes were missing or fused (Fig. 4B' and D'). Another pair-rule gene product Fushi-tarazu exhibited similar aberration (data not shown). In the same embryos with abnormal Eve pattern, the Kni and Hb domains appeared normal (Fig. 4B and D). However, when we examined the transcripts of *kni* by *in situ* hybridization, we found that the transcripts were less abundant in the mutant than in wild type (Fig. 4E and F).

To better examine whether Kni and Hb expressions were affected, we quantified the Kni and Hb distribution over total lateral embryonic area. We found that in mutant embryos the Kni domain was reduced whereas both the anterior and posterior Hb domains were increased. The reduction of Kni domain was consistent at both mRNA and protein levels (Fig. 5A). The Kr distribution also showed a slight compaction (Fig. 5C and data not shown). The reduction of *kni* expression domain observed in the *dAscl* mutant background could be a change in transcription or mRNA turnover.

To determine whether the abnormal embryonic patterning is due to the disruption of the more upstream regulators, we examined the expression of the maternal Bicoid (Bcd) and Nanos (Nos) proteins in the *dAcsl* maternal mutants. Bcd and Nos are respectively the anterior and posterior organizers of the body patterning (Driever and Nusslein-Volhard, 1988a, 1988b; Gavis and Lehmann, 1992; Lehmann and Nusslein-Volhard, 1991). The Bcd gradient and Nos expression appeared normal in *dAcsl* maternal mutant embryos (Fig. 6A, B, E, and F), except that the Bcd gradient seemed 'flatter' than in the wild type (Fig. 6C and D).

To better reflect the potential changes in Bcd gradient, we looked at the initial zygotic expression of Hb which is under the direct positive control of Bcd. The zygotic activation of Hb gives a relatively sharp boundary around nuclear Cycle 13 (Fig. 6G), which provided us an opportunity to compare the Hb expression in wild type and the *dAcsl* maternal mutant embryos. Consequently, we detected an expansion of Hb in the mutant embryos (Fig. 6G–I). Because the zygotic anterior Hb is required to set the anterior boundary of Kni abdominal stripe by its repressive action (Hulskamp et al., 1990),

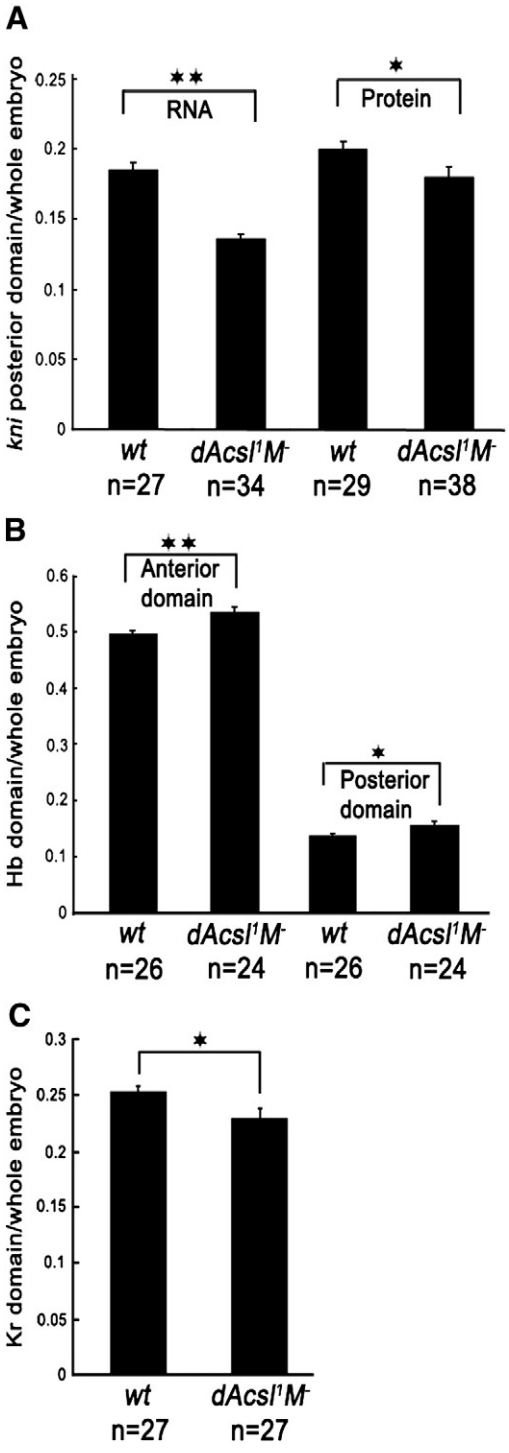

Fig. 5. The Kni expression domain was reduced whereas the Hb expression domain expanded in the *dAcsl* mutant embryos.(A) The area of the posterior Kni domain was normalized against the total lateral surface area of the embryo. (B) The areas of anterior or posterior Hb protein domains over total lateral surface area. (C) The areas of Kr protein domains over total lateral surface area. The $dAcsl^1M^-$ embryos were the progenies of heat-shocked females of *hsFLP; FRT42B* ovo^D/*FRT42B* $dAcsl^1$ mated to $dAcsl^1/CyO\ GFP$. These embryos included two zygotic genotypes which were undistinguishable at this stage. For each genotype, the total number of embryos examined is listed as '*n*'. $^*p<0.05$; $^{**}p<0.01$.

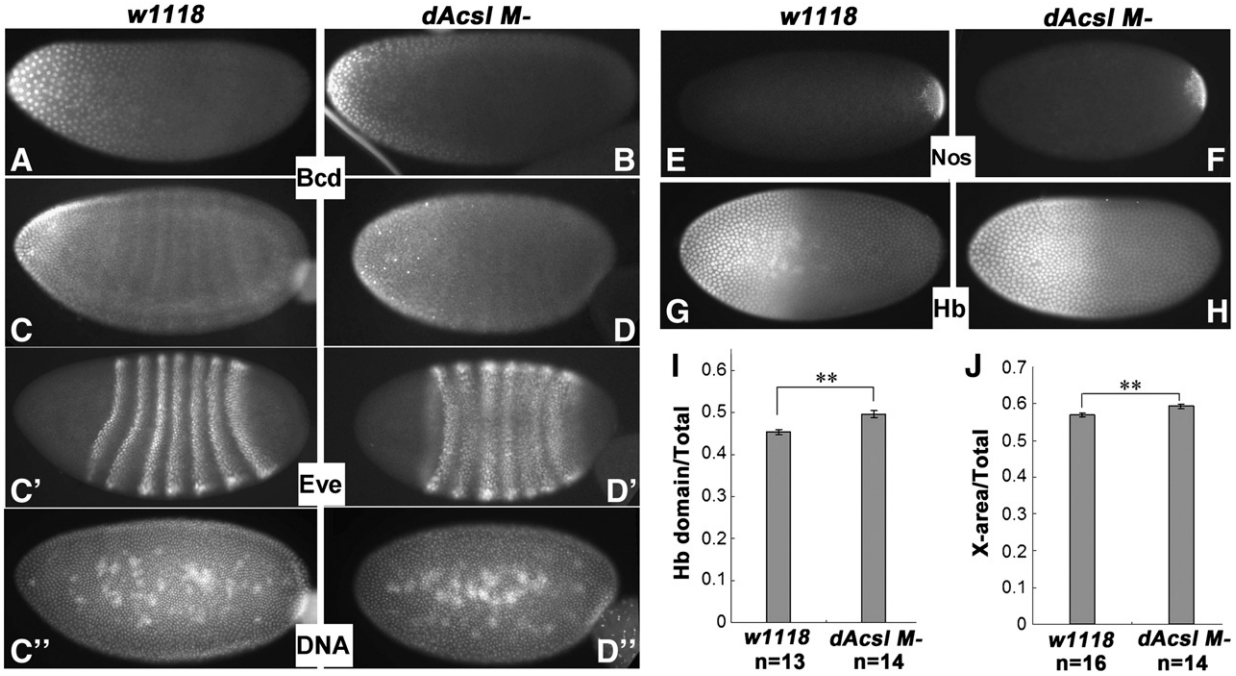

Fig. 6. Examination of *kni*'s upstream and downstream genes in the *dAcsl* maternal mutant embryos.The *dAcsl* M^- embryos were the progenies of heat-shocked females of *hsFLP; FRT42B ovo*^{D}*/FRT42B dAcsl*^{1} mated to *dAcsl*^{KO}*/CyO GFP*. These embryos included two zygotic genotypes which were undistinguishable before Stage 7. All embryos were immunostained and the images were taken at the same magnification. (A and B) Blastoderm embryos, ~Cycle 12–13. Embryos in panels C–C" or D–D" are the same ones co-stained for Bcd, Eve and DNA. (E and F) Nuclear cycle-1 embryos, determined by DNA staining (data not shown). (I) the anterior domain of Hb at nuclear cycle-13, shown in G and H, was measured and calculated against the total area. (J) 'X-area': the region anterior to the posterior stripe of *kni* transcripts shown in Fig. 4E and F. $^{**}p<0.01$.

correspondingly, the area anterior to the *kni* RNA stripe was expanded in the mutant than in the wild type (Fig. 6J). Taken together, the initial anterior expression of zygotic Hb was expanded and very likely shifted the position and reduced the expression of Kni posterior stripe.

Discussion

In the present study, we found that the fatty acid-mobilizing enzyme dAcsl is required for embryonic segmentation. The segmentation defects of *dAcsl* mutants resemble those of gap gene *kni*. The posterior domain of Kni transcripts or proteins was narrowed whereas the adjacent domains of another gap gene Hb correspondingly expanded in these mutants. Our findings reveal the connection between long-chain acyl-CoA synthetase and embryonic segmentation in *Drosophila.* We propose that *dAcsl* functions in embryonic segmentation by modulating gap gene expression.

The similarity in mutant phenotypes uncovers the possible link between this enzyme and *kni*. Although the strong genetic interaction exists between *dAcsl* and *kni*, two observations suggest that the function of *dAcsl* in segmentation seems not limited to *kni*. Firstly, the anterior Eve stripes were also affected in some mutant embryos where Kni is not expressed (Fig. 4D'). Secondly, *dAcsl* also genetically interacted with *Kr*. The alteration of gap gene expression is consistent with our genetic interaction results, in which *kni* or *Kr* reduction enhanced *dAcsl* segmentation defects whereas *hb* did not. Since the anterior zygotic Hb domain was expanded posteriorly in *dAcsl* mutants (Fig. 6G and H), this Hb shift could affect the anterior boundaries of both Kr and Kni domains (Hulskamp et al., 1990). Accordingly, we did expect to see certain degree of rescue of the *dAcsl* mutant phenotype when *hb* gene dosage was lowered by half. However, we did not observe an obvious effect which could simply be that one zygotic dosage of the Hb products along with the maternal contribution is enough to fulfill its normal function at this stage.

Also, the early zygotic expression of Hb was somehow expanded more posteriorly, indicating a spatial increase in response to Bcd activity. But we did not detect a corresponding increase of Bcd at protein levels though the Bcd gradient seemed less steep in the mutants (Fig. 6C and D). Additionally, we would have seen the effects not limited to *kni*-like phenotype if there were a posterior-ward shift due to a major change in Bcd. Further, because removing zygotic copy of *dAcsl* contributed ~4% more occurrence of segmentation defects than the maternal mutation alone (~11%), alteration in the gap gene functions cannot explain the defects developed post-zygotically unless dAcsl is also zygotically activated before cellularization.

How do we explain the gap gene-like phenotype in *dAcsl* mutants or how does *dAcsl* act on gap gene expressions/activities? One possibility is that the altered distribution of the upstream maternal factors since *kni* transcripts were spatially reduced in the *dAcsl* maternal mutants. There are abundant lipid droplets which participate in the vesicle transport and store maternal proteins in the early embryo (Cermelli et al., 2006; Welte et al., 1998). Since *dAcsl* is predicted as an enzyme mobilizing fatty acid and required for neutral lipids formation in larval tissues (Zhang et al., 2009), the aberration of lipid droplets formation was anticipated in *dAcsl* mutant embryos. Consequently, the distribution of certain maternal determinants may be affected because of the compromised membrane trafficking, altered protein localization, etc. If this hypothesis is true, then other mutations such as *Lsd2* which disrupt lipid droplets transport and neutral lipids storage in embryo (Gronke et al., 2003; Teixeira et al., 2003; Welte et al., 2005) should give similar phenotype as *dAcsl* mutations. However, we only observed very minor segmentation defects in *Lsd2* mutant cuticles (Table 1). Does the lipid storage decrease more in *dAcsl* than in *Lsd2* mutants? We examined the triglyceride levels in early embryos and could not detect a significant difference between the wild type and *dAcsl* or *Lsd2* mutant embryos (Fig. S1). The relationship between the lipid-droplets formation and embryonic segmentation remains elusive. Nonetheless, as a lipid metabolism-related enzyme, *dAcsl*'s effect in segmentation is specific and intriguing. However, the details of the connection between dAcsl and embryonic segmentation require more intensive investigations.

Supplementary materials related to this article can be found online at doi:10.1016/j.ydbio.2011.02.030.

Acknowledgments

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